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David Baltimore

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EXAMINER

RAMIREZ, DELIA M

ART UNIT

PAPER NUMBER

1652

MAIL DATE

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PAPER

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

## Office Action Summary

Application No.

10/656,531

Applicant(s)

BALTIMORE ET AL.

Examiner

Delia M. Ramirez

Art Unit

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**-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --**  
**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period **will** apply and **will** expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply **will**, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

### Status

- 1) ☒ Responsive to communication(s) filed on 05 July 2007.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

### Disposition of Claims

- 4) ☒ Claim(s) 21,28,40,43,99-104,106-113 and 119-143 is/are pending in the application.
- 4a) Of the above claim(s) 43,109-113 and 119-143 is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 21,28,40,99-104 and 106-108 is/are rejected.
- 7) ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

### Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on \_\_\_\_\_ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.  
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).  
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

### Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some \* c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\* See the attached detailed Office action for a list of the certified copies not received.

### Attachment(s)

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☒ Information Disclosure Statement(s) (PTO/SB/08)  
Paper No(s)/Mail Date 9/22/06.
- 4) ☐ Interview Summary (PTO-413)  
Paper No(s)/Mail Date: \_\_\_\_\_.
- 5) ☐ Notice of Informal Patent Application
- 6) ☐ Other: \_\_\_\_\_.

## **DETAILED ACTION**

### ***Status of the Application***

Claims 21, 28, 40, 43, 99-104, 106-113 and 119-143 are pending.

Applicant's election without traverse of Group I, claims 21, 28, 40, 99-104, 106-108 drawn to a vector and a mammalian cell comprising a nucleic acid encoding a chimeric nuclease and a nucleic acid comprising a repair substrate, as submitted in a communication filed on 7/5/2007 is acknowledged.

At this time, no product claim is found allowable. Therefore the restriction requirement between product and process claims can be properly maintained.

New claims 127-143 are directed to a non-elected invention, i.e., method claims. Claims 43, 109-113 and 119-143 are withdrawn from consideration as being directed to a non-elected invention. Claims 21, 28, 40, 99-104 and 106-108 are at issue and are being examined herein.

Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

### ***Information Disclosure Statement***

1. The information disclosure statement (IDS) submitted on 9/22/2006 is acknowledged. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

### ***Priority***

2. Acknowledgment is made of a claim for domestic priority under 35 U.S.C. 119(e) to provisional application No. 60/408,454 filed on 09/05/2002, 60/419,341 filed on 10/17/2002, and 60/484,788 filed on 07/03/2003.

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3. A single vector comprising a nucleic acid encoding a chimeric nuclease and a repair substrate was first indicated as a preferred embodiment in provisional application 60/484,788 filed on 07/03/2003.

***Claim Rejections - 35 USC § 112, First Paragraph***

4. The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

5. Claims 21, 28, 40, 99-104, 106-108 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 21, 99-102 are directed in part to vectors comprising nucleic acids encoding a genus of chimeric nucleases wherein said chimeric nucleases comprise a genus of DNA binding domains which recognize a genus of DNA targets. Claims 28, 40, 103-104, 106-108 are directed in part to mammalian cells comprising the vectors described above or the chimeric nucleases described above.

In *University of California v. Eli Lilly & Co.*, 43 USPQ2d 1938, the Court of Appeals for the Federal Circuit has held that “A written description of an invention involving a chemical genus, like a description of a chemical species, ‘requires a precise definition, such as by structure, formula, [or] chemical name,’ of the claimed subject matter sufficient to distinguish it from other materials”. As indicated in MPEP § 2163, the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant,

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identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show that Applicant was in possession of the claimed genus. In addition, MPEP § 2163 states that a representative number of species means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.

In the instant case, the claims encompass an extremely large genus of DNA binding domains which can recognize any DNA target or an extremely large genus of nucleic acids encoding said DNA binding domains. The genus of proteins/nucleic acids encompassed by the claims is structurally unrelated. While the specification discloses Zinc finger DNA binding domains that recognize a specific sequence within a nucleic acid encoding a variant of the green fluorescent protein (GFP) and the prior art teach a few examples of Zinc finger DNA binding domains that recognize a limited number of DNA targets, neither the specification nor the art teach the structural features required in a DNA binding domain such that it can recognize any DNA target. The specification fails to teach how to customize a DNA binding domain such that it can recognize a particular DNA target.

While one could argue that the disclosure of a Zinc finger DNA binding domain that recognizes a particular sequence (5' GGGGAAGAA 3' or 5' GCGTGGGCG 3') within a nucleic acid encoding a GFP variant, as well as the teachings of the prior art, provide adequate description for all the members of the genus recited, it is noted that the art teaches several examples of how even small changes in structure can lead to changes in target recognition. For example, Berglund et al. (Biochemistry 36:11188-11197, 1997) teach how three amino acid changes in the DNA binding domain of the glucocorticoid receptor (GR) result in the DNA binding domain of GR to recognize a different DNA response element (page 11188). Therefore,

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since (a) minor structural changes may result in changes affecting DNA recognition, (b) there is no additional information correlating structure with the recognition of a particular DNA target, and (c) there is no teaching or suggestion as to how to modify the Zinc finger DNA binding domains provided in the specification such that they can recognize any DNA target, one cannot reasonably conclude that the DNA binding domain provided in the specification or those known in the art are representative of the structures of all the DNA binding domains/nucleic acids required by the claims.

Due to the fact that the specification only discloses a single species of the genus, and the lack of description of any additional species by any relevant, identifying characteristics or properties, one of skill in the art would not recognize from the disclosure that Applicant was in possession of the claimed invention.

6. Claims 21, 28, 40, 99-104, 106-108 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for (1) a vector that comprises a nucleic acid encoding a chimeric nuclease, wherein said chimeric nuclease comprises a Zinc finger DNA binding domain that recognizes 5' GGGGAAGAA 3' or 5' GCGTGGGCG 3', and (2) an isolated mammalian cell that comprises said vector or said chimeric nuclease, does not reasonably provide enablement for (A) a vector that comprises a nucleic acid encoding a chimeric nuclease, wherein said chimeric nuclease comprises any DNA binding domain, or (B) a non-isolated mammalian cell comprising said vector or said chimeric nuclease. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required are summarized in *In re Wands* (858 F.2d 731, 737, 8 USPQ2d 1400 (Fed. Cir. 1988)) as follows:

1) quantity of experimentation necessary, 2) the amount of direction or guidance presented, 3) the

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presence and absence of working examples, 4) the nature of the invention, 5) the state of prior art, 6) the relative skill of those in the art, 7) the predictability or unpredictability of the art, and 8) the breadth of the claims. The factors which have lead the Examiner to conclude that the specification fails to teach how to make and/or use the claimed invention without undue experimentation, are addressed in detail below.

***The breath of the claims.*** Claims 21, 28, 40, 99-104, 106-108 are so broad as to encompass (1) vectors encoding chimeric nucleases wherein the chimeric nucleases comprise a DNA binding domain which can recognize any DNA target, and (2) mammalian cells comprising said vectors or said chimeric nucleases. The enablement provided is not commensurate in scope with the claims due to the extremely large number of DNA binding domains/nucleic acids of unknown structure recited in the claims. In the instant case, the specification enables (1) a vector that comprises a nucleic acid encoding a chimeric nuclease, wherein said chimeric nuclease comprises a Zinc finger DNA binding domain that recognizes 5' GGGAAGAA 3' or 5' GCGTGGGCG 3', and (2) an isolated mammalian cell that comprises said vector or said chimeric nuclease.

With regard to claims 28, 40, 103-104, 106-108, it is noted that the specification contemplates the vectors of the invention to be used for gene therapy, specifically gene targeting of defective genes associated with disease. See Background and Summary of the Invention sections. Therefore, in its the broadest reasonable interpretation, claims 28, 40, 103-104, 106-108 are directed not only to isolated host cells but also to host cells within a transgenic multicellular organism (i.e., non-isolated), including humans. The enablement provided is not commensurate in scope with the claims due to the extremely large number of transgenic multicellular organisms comprising the cells encompassed by the claims which the specification fails to teach how to generate or how to use, as well as the lack of information as to how to successfully deliver the claimed vector to a patient in order to obtain the desired DNA replacement. In the instant case,

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the specification enables an isolated host cell comprising a vector comprising a nucleic acid encoding a chimeric nuclease that comprises a DNA binding domain that recognizes 5' GGGGAAGAA 3' or 5' GCGTGGGCG 3', or an isolated host cell comprising said chimeric nuclease.

***The amount of direction or guidance presented and the existence of working examples.***

The specification discloses a vector comprising a nucleic acid encoding a chimeric nuclease wherein the chimeric nuclease comprises a Zinc finger DNA binding domain that recognizes 5'GGGGAAGAA 3' or 5' GCGTGGGCG 3', and isolated mammalian host cells comprising said vector or said chimeric nuclease, as working examples. However, the specification fails to provide any clue as to (1) the structural elements required in any nucleic acid encoding a DNA binding domain that can recognize any DNA target, or the structural elements required in said DNA binding domain, or (2) the structural features in the DNA binding domains disclosed in the specification which are required to recognize any DNA target.

With regard to claims 28, 40, 103-104, 106-108, while the specification discloses that the vectors of the invention can be used to transform isolated host cells, the specification also discloses that the vectors of the invention can be used for gene therapy by targeting specific genetic mutations known to be associated with disease and replacing the mutated gene. There are no working examples or specific methods disclosed showing a transgenic animal comprising said vector, or expressing the chimeric nuclease. Also, there are no working examples or specific methods disclosed showing how to deliver the claimed genus of nucleic acids to human tissues such that the claimed nucleic acids can be used for therapeutic purposes as suggested.

***The state of prior art, the relative skill of those in the art, and the predictability or unpredictability of the art.*** The nucleotide sequence of the coding region of a polynucleotide encoding a protein determines the structural and functional properties of that protein. In the instant case, neither the specification nor the art provide a correlation between structure and the

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ability to recognize any DNA target such that one of skill in the art can envision the structure of a DNA binding domain that can recognize the desired DNA target. In addition, the art does not provide any teaching or guidance as to the structural elements in the DNA binding domain provided by the specification or those known in the art required to recognize any DNA target.

Furthermore, while the argument can be made that the genus of DNA binding domains required is enabled due to the fact that there are several DNA binding domains known in the art, the art also teaches that small structural variations in a protein can affect function. The art clearly teaches that changes in a protein's amino acid sequence to obtain the desired activity without any guidance/knowledge as to which amino acids in a protein are required for that activity is highly unpredictable. At the time of the invention there was a high level of unpredictability associated with altering a polypeptide sequence with an expectation that the polypeptide will maintain the desired activity. For example, Branden et al. (Introduction to Protein Structure, Garland Publishing Inc., New York, page 247, 1991) teach that (1) protein engineers are frequently surprised by the range of effects caused by single mutations that they hoped would change only one specific and simple property in enzymes, (2) the often surprising results obtained by experiments where single mutations are made reveal how little is known about the rules of protein stability, and (3) the difficulties in designing *de novo* stable proteins with specific functions. The teachings of Branden et al. are further supported by the teachings of Berglund et al. who describe the modification of three amino acids in the DNA binding domain of the glucocorticoid receptor (GR) and how this results in the recognition of a different DNA target. Further support is found in Porteus et al. (Nature Biotechnology 23(8):967-973, 2005), who teach that Zinc fingers (DNA binding domains) require more refining to better discriminate between the gene target of choice and related off-target binding sites and assert that such refining will require further research into the nature of the recognition process, which is an area that has been studied by structural biologists for decades and is not trivial (page 972, left column, first full paragraph).

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Although the prior art teaches the potential of chimeric nucleases comprising zinc finger DNA binding domains for homologous recombination *in vivo*, such as in gene targeting, whether this can actually be carried out in primary cells or in an *in vivo* setting is unpredictable. Porteus et al. (Nature Biotechnology 23(8):967-973, 2005) teach that Zinc finger nucleases (ZFNs) have been primarily applied to transformed mammalian cell lines that are relatively resistant to apoptotic stimuli, but not primary cells that are more sensitive to DNA damage (see page 971, right column, Future Directions). Porteus et al. points out that one of the challenges of using ZFNs in primary cells is the delivery method, and indicates that it is unknown whether the techniques that have been used in cultured cell lines will work in primary cells or whether other methods, such as viral delivery or direct microinjection will be better (page 972, left column, second to last paragraph).

With regard to DNA delivery and expression in human tissues, the art teaches the high unpredictability of delivering DNA to human tissues and achieving the desired expression. For example, Phillips (J. Pharm. Pharmacology 53:1169-1174, 2001) teaches that the major challenges in gene therapy have been delivery of DNA to target cells and duration of expression (Abstract). According to Phillips, the problem regarding gene therapy is twofold in that (1) a system must be design to deliver DNA to a specific target while preventing degradation within the body, and (2) an expression system must be built into the DNA construct to allow the target cell to express the protein at therapeutic levels for a determined amount of time (page 1170, left column, lines 7-15). Gardlik et al. (Med. Sci. Monit. 11(4):RA110-121, 2005) teach that (1) while there are a number of methods known for delivery of DNA, there is no clear ideal delivery system (RA119, last paragraph), and (2) the main problem in gene therapy lies in the secure and efficient delivery of genes into target cells and tissues (RA110, Summary).

Urnov et al. (Nature 435:646-651, 2005) teach that while human T cells may be targeted by using a designed zinc finger nuclease in culture, they also indicate that the application of this

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technology to human cells in the clinic requires extensive study of ZFN safety in appropriate model systems (page 650, right column). Urnov et al. also teach that some of the limitations with regard to the use of ZFNs in the clinic are (1) successful delivery of DNAs encoding ZFNs and donor DNA, and (2) the potential immunogenicity of ZFNs. Porteus et al. also point out that the optimization of ZFN design must address two key questions: specificity and cytotoxicity (page 971, right column, last paragraph). Porteus et al. further indicate that future work will be needed to translate the in vitro findings to in vivo applications and to determine whether ZFNs create undesired genomic instability (Abstract). Therefore, based on the teaching of the current art, it is unpredictable whether the entire genus of non-isolated mammalian cells claimed is enabled.

***The quantity of experimentation required to practice the claimed invention based on the teachings of the specification.*** While methods of generating or isolating variants of a nucleic acid were known in the art at the time of the invention, it was not routine in the art to screen by a trial and error process for all the polynucleotides which would encode a DNA binding protein and determine which ones would recognize the desired DNA target. In the absence of (1) a rational and predictable scheme for selecting nucleic acids most likely to encode a protein having the desired activity (i.e., recognition of a specific DNA target), and/or (2) a correlation between structure and the desired activity, one of skill in the art would have to test an essentially infinite number of polynucleotides to determine which ones encode proteins having the ability to recognize the desired DNA target. While enablement is not precluded by the necessity for routine screening, if a large amount of screening is required, as is the case herein, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed so that a reasonable number of species can be selected for testing. In view of the fact that such guidance has not been provided in the instant specification, it would require undue experimentation to enable the full scope of the claims. Furthermore, given the teachings of the art regarding the unpredictability of delivering and expressing DNA in human

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tissues, and the unpredictability associated with the delivery and expression of DNA encoding ZFNs *in vivo*, as well as the lack of guidance provided by the specification, it would have required undue experimentation to engineer any transgenic mammalian organism comprising the recited cells.

Therefore, taking into consideration the extremely broad scope of the claims, the lack of guidance, the amount of information provided, the lack of knowledge about a correlation between structure and function, the high degree of unpredictability of the prior art in regard to (a) structural variability and its effect on function, (b) generation of transgenic mammalian organisms comprising the recited cells, and (c) delivery and expression of DNA in human tissues, one of ordinary skill in the art would have to go through the burden of undue experimentation in order to practice the claimed invention. Thus, Applicant has not provided sufficient guidance to enable one of ordinary skill in the art to make and use the invention in a manner reasonably correlated with the scope of the claims.

### ***Claim Rejections - 35 USC § 103***

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

8. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later

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invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

9. Claims 21, 28, 40, 99-104, 106-108 are rejected under 35 U.S.C. 103(a) as being unpatentable over Choulika et al. (U.S. Publication No. 20020107214, U.S. Application No. 10/917295 filed on 7/27/2001; cited in the IDS) in view of Bibikova et al. (Molecular and Cellular Biology 21(1):289-297, 2001; cited in the IDS) and further in view of Takeuchi et al. (Biochemical and Biophysical Research Communications 293:953-957, 2002).

Choulika et al. teach a chimeric nuclease comprising the DNA binding domain of a I-Sce I nuclease and the cleavage domain of a FokI nuclease [paragraph [0042]]. Choulika et al. also teach vectors comprising a nucleic acid encoding the chimeric nuclease and target DNA (paragraph [0044]). The target DNA of Choulika et al. comprises DNA homologous to the region surrounding the site to be targeted, and (2) DNA which repairs the specific sequence of interest upon recombination between the targeting DNA and the chromosomal DNA (paragraph [0026]). Choulika et al. discloses viral vectors (paragraph [0045]), inducible promoters (paragraph [0046]), and isolated mammalian cells comprising said vectors (paragraph [0052]). Choulika et al. do not disclose the use of a nuclear localization signal or a vector comprising a DNA encoding a second chimeric nuclease. Bibikova et al. teach the use of two chimeric nucleases with different binding specificities (Zinc finger DNA binding domains) which together collaborate to stimulate recombination when their individual sites were appropriately placed (Abstract; Materials and Methods, Enzymes). Bibikova et al. also teach the direct injection of these chimeric nucleases and DNA substrates for recombination directly into the nucleus of oocytes (Materials and Methods, Oocyte injections). Bibikova et al. do not teach a single vector comprising DNA encoding the two chimeric nucleases and the DNA substrate. Takeuchi et al. teach a vector comprising DNA encoding Flp recombinase linked to a nuclear localization signal to increase the efficiency of the recombination process (page 954, right column, Results).

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Takeuchi et al. do not teach a vector comprising DNA encoding one or two chimeric nucleases and a DNA substrate.

Claims 21, 99-101, 103-104 are directed in part to a viral vector comprising a DNA encoding a chimeric nuclease, wherein said chimeric nuclease comprises a DNA binding domain, a nuclear localization signal, and a FokI cleavage domain, wherein the DNA encoding the chimeric nuclease is operably linked to an inducible promoter, and wherein said viral vector further comprises a DNA repair substrate. Claim 102 is directed in part to a vector comprising DNA encoding two chimeric nucleases, wherein said chimeric nucleases form a heterodimer, wherein said chimeric nucleases comprise a DNA binding domain, a nuclear localization signal and a cleavage domain, and wherein said vector further comprises a DNA repair substrate. Claims 28, 40, 107-108 are directed in part to isolated mammalian cells transformed with the vector of claim 21 as described above. Claim 106 is directed in part to an isolated mammalian cell comprising a chimeric nuclease, wherein said chimeric nuclease comprises a DNA binding domain, a nuclear localization signal, and a cleavage domain, wherein the DNA binding domain comprises a Zinc finger, and wherein said cell further comprises a DNA repair substrate.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to further add a DNA encoding a nuclear localization signal to the vector of Chouluka et al. and transform the isolated mammalian cells of Chouluka et al. with said vector. Also, it would have been obvious to one of ordinary skill in the art at the time the invention was made to create a vector encoding the two chimeric nucleases of Bibikova et al. and the DNA substrate of Bibikova et al., wherein the chimeric nucleases comprise a nuclear localization signal. A person of ordinary skill in the art is motivated to add a DNA encoding a nuclear localization signal to the vector of Chouluka et al. in view of the teachings of Takeuchi et al., who teach that adding a nuclear localization signal enhances recombination. Also, a person of ordinary skill in the art is motivated to create a vector comprising DNA encoding the two chimeric nucleases of Bibikova et

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al., nuclear localization signals, and further comprising the DNA substrate, for the benefit of delivering to an isolated mammalian cell all the necessary components for recombination in a single vehicle and to enhance recombination as taught by Takeuchi et al. One of ordinary skill in the art has a reasonable expectation of success at making the vectors and transforming isolated mammalian cells with said vectors since (1) Chouluka et al. and Takeuchi et al. teach the construction of vectors comprising DNA encoding chimeric nucleases and nuclear localization signals, respectively, (2) Bibikova et al. teach vectors encoding each of the chimeric nucleases comprising Zinc finger DNA binding domains, and (3) transformation of isolated mammalian cells with vectors is well known and widely used in the art. Therefore, the invention as a whole would have been prima facie obvious to a person of ordinary skill in the art at the time the invention was made.

### *Conclusion*

10. No claim is in condition for allowance.

11. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

12. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Delia M. Ramirez whose telephone number is (571) 272-0938. The examiner can normally be reached on Monday-Friday from 8:30 AM to 5:00 PM. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. Nashaat Nashed can be reached on (571) 272-0934. Any inquiry of a general nature or relating to the

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status of this application or proceeding should be directed to the receptionist whose telephone number is (571) 272-1600.

/Delia M. Ramirez/

Delia M. Ramirez, Ph.D.  
Primary Patent Examiner  
Art Unit 1652

DR  
May 12, 2008